POLARITY AND BILATERALITY OF THE ANNELID EGG. EXPERIMENTS WITH CENTRIFUGAL FORCE.[1]

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In a previous paper (Lillie, '06) I attempted to show that the direction of polarity of the egg of *Chætopterus* is not modified by any experimental redistribution of the visible elements, nucleus and granules, and I therefore concluded that polarity is a property of the ground substance of the protoplasm. In the present paper I propose to examine the grounds for this statement, to attempt to show that bilaterality comes in the same category, and to examine the conceptions of polarity and organization that naturally result.

I. Polarity.

Polarity manifests itself first in the egg of *Chætopterus* when the ovogonium becomes an ovocyte and takes its place in the ovarian epithelium. It has then a free pole and an attached pole, which have different physiological relations and exhibit different morphogenic activities. When the egg becomes free from the epithelium it is found that the original free pole becomes the animal pole in development, and the attached pole becomes the vegetative pole. The developmental processes take place with reference to these poles; thus the various granules of the egg rearrange themselves in a definite polarized fashion, the polar bodies have an absolutely determined relation to polarity, cleavage takes place and the various organs arise in definite topographical relations to the polarity.

In the paper already referred to I showed that if the egg is centrifuged with sufficient force the result is to separate the protoplasm into three strata, viz.: a central grayish cap, an intermediate hyaline or clear band which contains the nucleus

[1] The substance of a part of this paper was presented before the joint session of the American Society of Zoölogists, and Section F—Zoölogy—of the American Association for the Advancement of Science, held in Chicago, December, 1907. (An abstract was printed in *Science*, Vol. XXVIII., No. 702, pp. 905–907, June 12, 1908.)

or spindle, and a distal yellow hemisphere which contains the larger granules known as yolk (Fig. 1). The results prove that the eggs are not oriented in the centrifuge with reference to the original polarity, and therefore the axis of stratification bears all

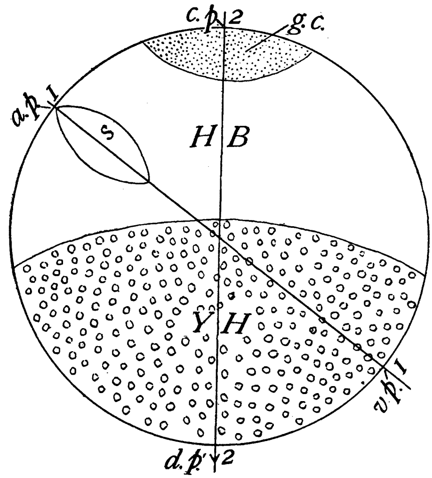

FIG. 1. Diagram of a centrifuged egg of *Chætopterus*. *a.p.*, animal pole; *c.p.*, central pole, or pole turned towards the axis of the centrifuge; *d.p.*, distal pole, or pole turned away from the axis of the centrifuge; *g.c.*, gray cap; *H.B.*, hyaline or clear band; *s.*, maturation spindle; *v.p.*, vegetative pole; *Y.H.*, yellow, yolk-bearing, or distal hemisphere; *1–1*, primary axis; *2–2*, secondary axis.

possible relations to the polar axis of the egg. We may call the original polar axis the primary axis, and the line through the center of the egg in the direction of the centrifugal force the secondary axis (Fig. 1).

If such eggs are fertilized the polar globules of different eggs form at any point of the surface with reference to the secondary axis. Cleavage takes place with reference to the axis defined by the polar globules, as in the normal egg, and is approximately normal in form. It therefore follows that the distribution of yolk and other substances induced by the centrifuge is substantially immaterial so far as the form of cleavage is concerned. The effect of abnormal distribution of yolk and other substances on the differentiation of organs is not considered here.

On the basis of these results I concluded that the polarity of the ovum had not been affected by centrifuging, and that the

polar bodies appear in the same position that they would have normally, that is, in the primary axis; in other words, that no redistribution of the granules or nucleus affects the direction of polarity of the egg. If this be true, then the final conclusion stated in that paper, that polarity is a function or property of the ground substance of the protoplasm, certainly follows.

This conclusion is of fundamental importance for the entire theory of development, and its basis therefore requires the most careful examination. One thing is clear at the start, viz.: that this point of view affords a complete explanation of the phenomena, because the egg is polarized prior to centrifuging, and the centrifugal force acts in all possible directions with reference to the polar axis;[1] and this agrees with the fact that the polarity that appears after centrifuging also lies in all possible directions with reference to the axis of stratification. The question is simply this: Is the polarity as marked by the polar bodies and cleavage planes the same polarity in the normal egg and after centrifuging? Does any other hypothesis agree so well with the observed facts, and if so, is it for any sufficient reason to be preferred to this hypothesis?

An alternative hypothesis can lie only in one direction, viz.: that polarity is determined by any point on the surface of the egg to which the maturation spindle happens to go after cen-

[1] The proof of this statement is as follows: In the stage used for the experiments (except where otherwise stated) the first maturation spindle is fixed at the periphery at the stage of the mesophase. Its outer pole marks the center of the animal pole; this point is also characterized by an entire absence of the ectoplasm, which covers the remainder of the surface of the egg as described in a previous paper ('06). If sections of centrifuged eggs are examined, the maturation spindle is found in about half of the eggs still attached to the surface, at any point in the hemisphere containing the hyaline band. At first sight this would indicate that the eggs oriented themselves in the centrifuge to a slight extent, though it is clear that the animal pole is in some cases 90° removed from the ends of the secondary axis. But the fact that fixed spindles are not found in the distal (yolk-laden) hemisphere after centrifuging has another explanation: about half of the eggs after centrifuging have free spindles (*i. e.*, not attached to the surface), which always lie in the hyaline band, and these represent those eggs whose original animal pole lay in the distal hemisphere in the centrifuge. (Cf. Fig. 8, *C* and *D.*) The inrush of yolk into the distal hemisphere has torn the spindles loose from the periphery, and their specific gravity has carried them into the hyaline band; this interpretation of the free spindles is proved by the fact that many of them migrate into the distal hemisphere later in the process of forming the polar bodies (Fig. 2).

trifuging. Now, assuming that there is no polarity of the ground substance, as this hypothesis requires, the spindle would naturally follow the shortest path to the surface, for this would usually be the path of least resistance. In other words, the position given the nucleus or the spindle by the centrifugal force would determine the polarity in all cases, because its position is always excentric. However, this is not the case, as the following facts demonstrate :

1. Most of the experiments on the egg of *Chætopterus* were made before fertilization at the stage of the mesophase of the first maturation spindle. All the ripe eggs of this animal attain this stage without fertilization, and the spindle comes to rest and remains at the mesophase unless the egg be fertilized or otherwise effectively stimulated (Fig. 4). The spindle is attached to the surface and its axis is in line with the axis of the egg. If the direction of the centrifugal force is such that the spindle lies in the central hemisphere, *i. e.*, in the half of the egg towards the post, it may remain attached, but is often detached from the surface, and in such cases it always lies in the clear band. If the spindle pole of the egg lies in the distal hemisphere during centrifuging, it is always detached from the surface and comes to rest in the clear band. The result therefore is always the same so far as the position of the spindle is concerned : the spindle lies invariably in the clear band after centrifuging, either attached to the surface, or free.

Nevertheless, the polar bodies may form at any point in the yolk hemisphere, as is demonstrated by study of the living eggs, and also by sections of series of eggs preserved during the maturation period after centrifuging. Figures of such living eggs are given in the paper already referred to, and a section of such an egg is reproduced here (Fig. 2). When one considers the resistance that has to be encountered by the spindle in making its way through the densely packed yolk-granules of the distal hemisphere, and remembers that a shorter path to the surface through hyaline protoplasm was open, it is obvious not only that the spindle does not follow the shortest path to the surface, but also that a factor of great intensity has been in operation.

I stated in my previous paper ('06) that the formation of polar

globules in the yolk hemisphere is rarer than their formation in the hyaline hemisphere, and was inclined to regard this as evidence that there was a tendency for the eggs to orient themselves in the centrifuge with the vegetative pole away from the center. But I no longer believe that this is the case. The relative rarity of maturation at the distal pole of centrifuged eggs must be due to a different cause, and this is the great resistance of the yolk

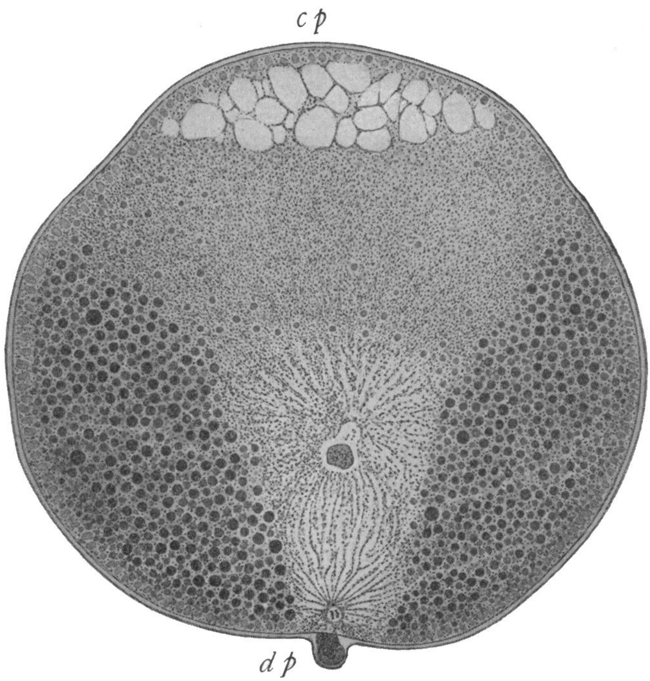

FIG. 2. Egg of *Chætopterus* centrifuged 2,300 revolutions in one minute at a radius of 13 cm., 3 : 57 P. M., June 27, 1906; fertilized 3 : 58 P. M., killed 4 : 27 P. M. The formation of the polar globules has taken place at the distal pole. The maturation spindle migrated through the entire yolk-mass in the distal hemisphere to reach this position. The sperm-nucleus surrounded by radiations is seen a short distance above the egg-nucleus. The vacuolated area above is the gray cap. *c.p.*, central pole (secondary axis); *d.p.*, distal pole (secondary axis).

mass to penetration by the spindle. This conclusion is clearly demonstrated by study of series of sections of eggs preserved at regular intervals after centrifuging and fertilizing, which show that many such eggs fail to form the polar bodies at all, or are much delayed, or undergo their maturation divisions within the egg.

2. An even more conclusive argument is afforded by centrifuging ovocytes with intact germinal vesicle. As shown in the paper already referred to, the large germinal vesicle passes to the central pole; it is the lightest constituent of the egg. If, therefore, the position of the nucleus determines polarity, the central pole should become the animal pole in such cases; the polar bodies should form here. I made a number of experiments on this subject in 1906 and 1908. The difficulties found in interpreting these experiments are: (*a*) that the stratification induced by low centrifugal powers is relatively slight in the stage with intact germinal vesicle, apparently owing to greater vicosity of the ground substance at this stage, and (*b*) that it does not persist to the time of formation of the polar bodies, owing to the process of polarization of granules that follows the rupture of the germinal vesicle, which I described in a previous paper. However, by following individual eggs in which the position of the centrifuged germinal vesicle is known, the relation of the polar bodies to the position of the germinal vesicle can be readily ascertained. It is then found that there is no fixity of relation whatever; in other words, the maturation spindle, which forms at the site of the germinal vesicle, may migrate through the entire diameter of the egg to become fixed at the opposite pole, or perform any lesser migration. The same thing is demonstrated if the eggs are submitted to very high centrifugal powers; under these circumstances the stratification persists up to maturation in some eggs to a sufficient degree to demonstrate positively that the polar bodies form in all positions with reference to the centrifuged position of the germinal vesicle.

Thus one cannot determine the place of formation of the polar bodies by simply throwing the nucleus to the surface of the egg.

A third argument may be based on comparative grounds. Very many cases are known in which polarity determines the position of the nucleus; the literature on fertilization is full of such cases. I know of no observations that tend to show determination of polarity by position of the nucleus.

The results, then, certainly demonstrate that polarity is not a result of the position of the nucleus or of any configuration of granules. It follows that it is a property of the ground sub-

stance; and when one considers carefully the data presented, one cannot avoid the conclusion that the direction of polarity is unmodified by the centrifugal powers employed. It must therefore depend on some configuration or heterogeneous physical or chemical properties of the ground substance established early in the history of the egg, and which is not essentially disturbed by centrifuging. This subject will be examined in the third part of the present paper.

The conclusion stated above was clearly presented in my paper of 1906. Since then Morgan (1908) has come to the same conclusion for the egg of *Arbacia*. In his last paper Morgan furnishes a satisfactory demonstration that polarity is unmodified in the egg of *Arbacia* by centrifuging, by showing that the micropyle which bears a constant relation to polarity in the normal egg has the same constant relations to the polarity of centrifuged eggs, as proved by formation of the micromeres and archenteron at the pole opposite the micropyle whatever the direction of the centrifugal force, and therefore whatever the position of the centrifugal zones and the form of the cleavage modified (in *Arbacia*) by these zones. I therefore take Morgan's result to be a complete confirmation of my original and present position.

The demonstration that the direction of polarity is unaltered by centrifuging involves the assumption that the configuration of the ground substance remains the same. In other words, that there is a definite architecture in the ground substanee, which is the basis of the localization pattern in normal development (see *Science*, N. S., XXVII., June 12, 1908). This also involves a new conception of protoplasmic streaming as seen in ova, for this must be interpreted as granule movement exclusively, and not actual flowing. Similarly, the movements produced by the centrifuge cannot be mass movements of entire protoplasmic areas, but only granule movements through the ground substance. In the third part of the present paper, I shall consider these conclusions with some care.

Observations on Nereis.

In the summer of 1908, I had the opportunity of comparing the eggs of *Nereis limbata* with reference to these points, and I

found that, just as in *Chætopterus*, the place of formation of the polar globules is independent of centrifugal stratification, and that the cleavage follows the polarity marked by the polar globules and is not modified in its direction by the position of the yolk arbitrarily imposed by the centrifuge. Morgan has shown the same thing for the lamellibranch, *Cumingia* (*Science*, Vol. XXVII., p. 496, 1908), so that now we have at least four forms in which this principle is firmly established.

II. Bilaterality.

The second main question that I propose is whether or not bilaterality comes under the same category as polarity. And this resolves itself into two questions: (1) Is bilaterality, like polarity, a function of the ground substance, and (2) When does it arise?

1. The first morphological evidence of bilaterality in the eggs of *Chætopterus* and *Nereis* is found in the first cleavage. The spindle takes an excentric position, and the cleavage is therefore unequal; the smaller cell is anterior and the larger cell posterior in position. The course of the entire development is thus determined not only with reference to the bilateral axis, but also with reference to the proportions of embryonic organs.

If the bilaterality were a result of a kind of equilibrium of the protoplasmic inclusions (nucleus and granules), it is clear that the variations in position of the nucleus and granules produced by centrifugal force should involve change of direction of bilaterality. If, on the other hand, bilaterality is a function of the ground substance, changes in the position of the nucleus and granules should not materially affect the direction of bilaterality, though it might conceivably modify the inequality of the cleavage which is our index of bilateral organization. However, if the normal proportions of the first two cells and the meridional direction of the cleavage plane are maintained in centrifuged eggs in spite of the abnormal distribution of granules between cells that would be thus induced, no other evidence would be necessary to show that the seat of bilateral organization is in the ground substance.

This is in fact the case, both in *Chætopterus* and *Nereis*: Whatever the distribution of yolk and granules induced by centrifuging,

with reference to the primary axis, the first cleavage is invariably truly meridional, and the proportions of the cells are usually normal or approximately so. In some cases the small cell is packed full of yolk granules, in other cases practically devoid of them; nevertheless, the first cleavage is substantially unaffected as to direction or proportions by the direction of stratification (see Lillie, 1906, p. 199).

2. Is the bilateral organization of the egg a property of the primary ovocyte like polarity, or does it arise in the course of development after rupture of the germinal vesicle? The evidence seems to me in favor of the latter proposition for the following reasons:

(*a*) If the ground substance is bilaterally organized before maturation there should be morphological evidence of it, as there is of the existing polarity. But there is none whatever; the structure of the egg is radially symmetrical in the plane at right angles to the polar axis until the close of maturation. After the union of the germ nuclei, the bilateral symmetry comes to expression slowly by granule movements that cause a preponderant distribution of the yolk granules on one side of the egg, which becomes the posterior side, and by corresponding displacement of the yolk-free protoplasm and spindle towards the opposite side of the egg, which becomes the anterior side. This is the case in both *Chætopterus* and *Nereis*, as well as in *Unio*. If the conditions causing such displacement existed prior to maturation, one would expect that they would become effective at an earlier period.

(*b*) In the summer of 1908 I found that it was possible to break up the eggs of *Chætopterus* into fragments of varying sizes by high centrifugal force. The protoplasm of this egg is much more fluid than that of any other egg I have tested; in consequence, the egg elongates even with relatively low centrifugal speed; and with very high speed the eggs break apart along the lines of stratification. Thus one obtains numerous perfectly hyaline pieces composed of the substance of the clear band, and others densely packed with yolk granules. The nucleus is almost invariably contained in the former kind, but it may occur in the latter, owing to the fact that at the time of centrifuging the matu-

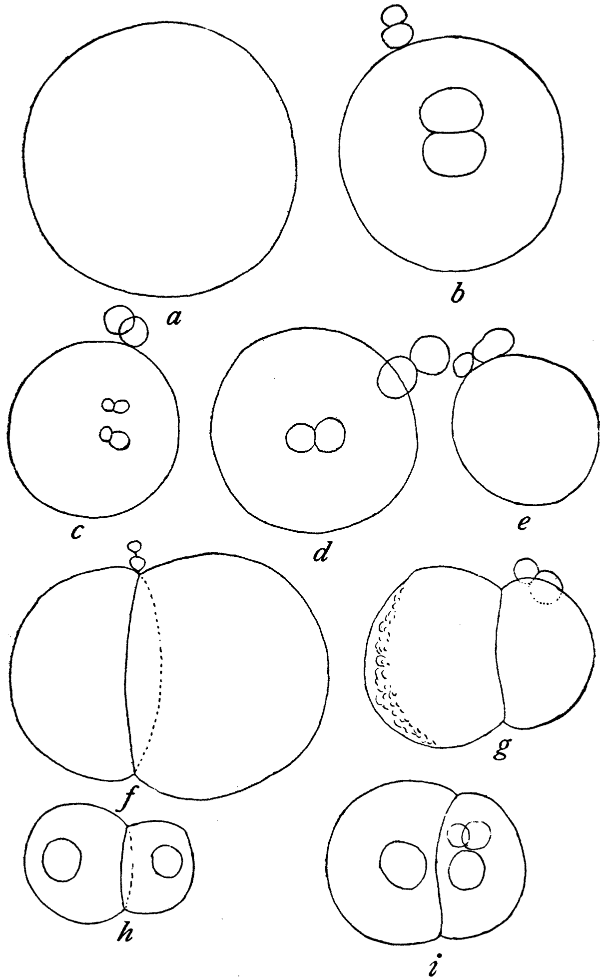

FIG. 3. Maturation and first division of hyaline fragments of the eggs of *Chætopterus*, obtained by centrifuging before fertilization. *a*, outline of normal egg for size-control ; *f*, outline of normal two-celled stage for size-control ; *b*, *c*, *d*, *e*, *g*, and *h*, hyaline fragments of various sizes from a single experiment ; *i*, a similar fragment from another experiment. Fragment *g* contains a small quantity of yolk.

The eggs break apart along the planes of stratification with a high centrifugal force, and further subdivision may occur so that the fragments are variable in size.

It will be noted that the polar globules are abnormally large in the fragments (cf. Fig. *f*, control). The proportions of the first two cells are, however, approximately normal, Figs. *g*, *h*, *i* (cf. *f*, control).

All figures drawn with the camera at a magnification of 780 diameters, and reduced one half.

ration spindle is attached to the surface, and may thus be separated off in the yolk-bearing end of the centrifuged egg, if it lies in a distal direction in the centrifuge and is not torn loose from its attachment before rupture of the egg takes place. But while the majority of large hyaline pieces contain the maturation spindle and form polar bodies after fertilization, this happens very rarely indeed in yolk-bearing pieces.

I was interested to determine if the cleavage of the hyaline pieces was unequal, and, if so, whether or not the proportions of the cells approximated the normal. This is indeed the case ; in most pieces taken before fertilization, that segment, the segmentation is unequal and in substantially the normal proportions. Seeing that the inequality of cleavage of the normal egg is an unfailing index of bilaterality, the same phenomenon in the parts must be interpreted in the same way.

The unequal cleavage of such hyaline parts absolutely devoid of yolk is a most striking phenomenon. There is great variation in the absolute size of such parts, but the cleavage remains proportional.

The parts vary not only in size, but also in the regions of the egg that they represent. The latter conclusion obviously follows from the fact that the direction of the centrifugal force is a matter of chance.

To show the strong tendency of pieces of the hyaline band to segment in normal proportions the following result may be cited. In experiment 10 *F*, August 18, 1908, a quantity of eggs taken from the female at 10 A. M. was allowed to stand in sea water without fertilization until 10 : 40 A. M. They were then centrifuged about 7,500 revolutions in one minute with a radius of 6 cm. and were thus broken up. The material was fertilized at 10 : 43 and allowed to develop to the two-celled stage, then fixed, stained and mounted entire in balsam. The impression of the preparation is that in practically every segmented hyaline piece the proportions of the cells are approximately normal. To remove the personal equation as far as possible, I marked a ring on the cover slip, and drew every segmented hyaline piece in it. Of 26 pieces so drawn, none had divided equally ; in four pieces of the 26 the small cell was proportionally larger than normal,

and the remainder were substantially normal in the relative sizes of the two cells, although the smallest piece was about one eighth the volume of the entire egg, and the others ranged in size up to about one third the bulk of the normal egg. The pieces in which the small cell was too large relatively were the largest pieces and contained some yolk.

Now if bilateral symmetry existed prior to fertilization, one would expect parts of the bilateral egg to vary greatly in the proportions of the first two cells, because a fractional bilaterality and not a whole bilaterality would exist in each part. I therefore conclude that bilaterality has developed subsequent to fertilization. And this conclusion must be true *for the parts* even if it were only a question of regulation of a preëxisting bilaterality (of which there is no evidence). But if bilaterality can develop in the parts there is no reason for assuming its prior existence in the whole ; and its origin must be regarded as a truly epigenetic process. Brachet makes a similar argument for the egg of the frog (*Roux's Archiv*, XXII., 3, 1906).

III. The Organization in the Ground Substance. Examination of the Conceptions of Polarity and Bilaterality.

The hypothesis that the nature and direction of polarity and bilaterality are unaltered by centrifuging involves the assumption that the arrangement of the ground substance is substantially unaltered. If it could be shown, for instance, that the segregation produced by centrifuging is a mass movement of the areas and zones of the protoplasm, and not simply a movement of granules through the ground substance, the entire organization of the egg would be altered, and there would be no reason why polarity should appear in the original direction rather than in any other direction.

I propose therefore to examine the thesis that the movements produced by centrifuging are purely granule movements, with the single exception of the nucleus or spindle. To do this it is necessary to compare minutely the structure of the egg both before and after centrifuging, using a fixed and determinate stage for such examination. Fortunately, the latter condition is a rela-

tively simple matter in the case of the egg of *Chætopterus*, because all ripe eggs reach such a stage in about twenty minutes after they are put in sea water, and undergo no further progressive development unless fertilized or otherwise suitably stimulated.

This stage may be described as follows (Fig. 4):

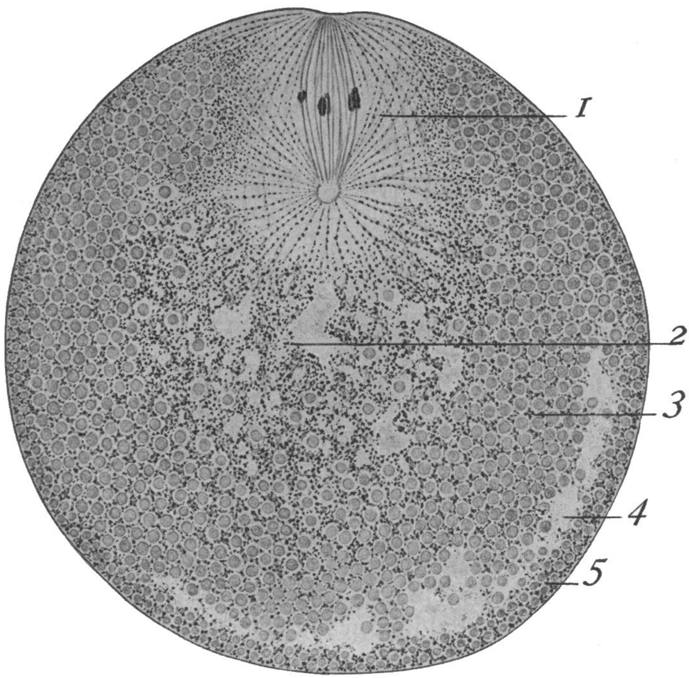

FIG. 4. Section of the egg of *Chætopterus* at the stage used for the experiments. See text for description.

The substance of the polarized egg shows the following areas and zones:

1. *The spindle area* lies at the animal pole and includes the spindle and the protoplasm in which the astral rays are developed. This area is predominantly basophile, but it may include a very few acidophile yolk granules. The astral rays may encroach slightly on (2) and (3).

2. *The central spongy area* lies near the center of the egg in the direct line of prolongation of the spindle. Its spongy appearance is due to paucity of granules and not to actual vacuoli-

zation of the ground substance. The basophile granules predominate and are arranged in a loose spongy network. This area is bounded by the spindle area on the animal pole side, and by the spherular zone (3) on the other sides. The boundary towards the spindle area is a little indefinite, fairly definite on the other sides. Its form is that of an indented sphere, the indentation being produced by the spindle area. Its position is about the same as that of the germinal vesicle before rupture.

Together, the spindle area and the central spongy area form an ovoidal mass, the small end of which reaches the surface at the animal pole, while the large end extends below the center of the egg.

The other zones of the egg are arranged concentrically to this mass, and are therefore open at the animal pole and thickest at the sides and vegetative end.

3. *The spherular zone* is so named because it contains the major part of the endoplasmic spherules or endoplasmic acidophile granules (yolk). In section, it appears as a very deep gourd-shaped crescent. The thin horns reach up above the level of the equatorial plate of the spindle.

4. *The intermediate spongy zone* lies between (4) and (5). In many eggs it is barely noticeable, in others it is very prominent. Like the central spongy zone, it is not a vacuolated portion of the ground substance, but it is characterized by paucity of granules, and so appears unstained in sections. It is a genuine structural feature as is proved by experiments, and also by the fact that it becomes more pronounced in eggs allowed to remain in the sea water (Fig. 5).

5. *The ectoplasmic zone* is the peripheral boundary of the egg, except that it is defective at the animal pole where the spindle area comes to the surface. It is characterized by the presence of spherules that differ in certain properties from those of the spherular zone (endoplasm), as described in detail in a previous paper.

In eggs allowed to stand in sea water without fertilization these areas become more strongly differentiated (Fig. 5), and the fact that the egg has a concentric as well as a polar organization stands out more strongly. The central spongy area now appears

to have a thick and dense wall in which the spherules of the spherular zone are especially aggregated. The intermediate spongy zone appears wider. The striking similarity of this con-

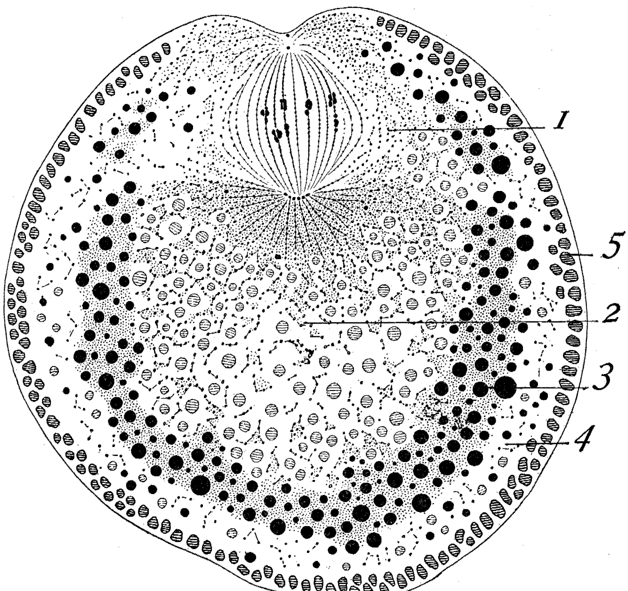

FIG. 5. Section of an unfertilized egg of *Chætopterus* that stood in sea water two hours and seven minutes before killing.

figuration to that produced by low centrifugal powers will be seen as the description proceeds.

We may now compare the pictures presented by eggs of this stage that have been centrifuged at different speeds: For this purpose we shall select first an egg that was centrifuged 1,150 revolutions in 31 seconds at a radius of 13 centimeters (Fig. 6). Comparison of a large number of eggs shows first that the position of the eggs in the centrifuge is a matter of chance and second that the segregation produced is essentially the same in whatever direction the centrifugal force may act with reference to the polar axis. We may therefore leave the question of direction of centrifugal force out of account. As already noted, the effect on the living egg is very striking: it appears stratified in the direction of action of the centrifugal force; at the central pole is a small gray cap, next to it a broad, clear or hyaline

band, and the distal hemisphere appears intensely yellow, and packed full of yolk-granules. The surface of apposition of the hyaline band and yellow hemisphere is not a plane surface, but decidedly convex towards the hyaline band.

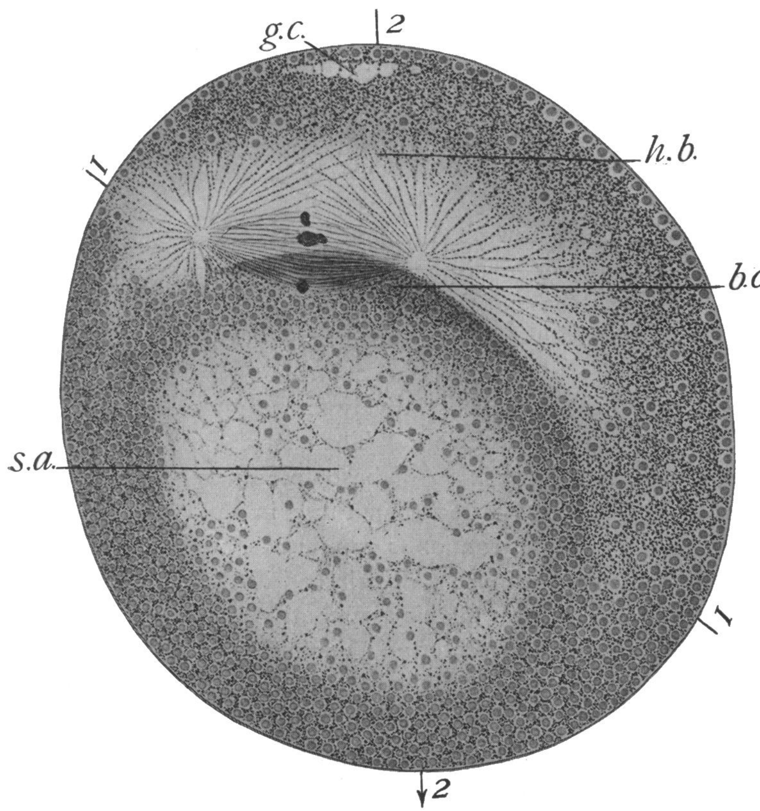

FIG. 6. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. The force was not sufficient to produce a very full segregation of the material of the gray cap. *1–1*, primary axis of the egg ; *2–2*, secondary axis. *b.c.*, basophile cap ; *g.c.*, gray cap ; *h.b.*, hyaline band ; *s.a.*, spongy area ; stained in thionin and orange *G*.

The gray cap is composed of fat globules, as is demonstrated by staining with osmic acid (Fig. 9) ; whether or not it includes the residual substance of the germinal vesicle as maintained in my former paper, I am not now prepared to say. In *Nereis*, in place of the gray cap, we have an aggregation of the large oil drops

characteristic of this egg. The hyaline band contains the small basophile granules, and the distal hemisphere the large acidophile granules (yolk-spheres). In sections stained in thionin and orange *G* the hyaline band is bright blue and very finely granular; the distal hemisphere is orange, owing to the affinity of the large yolk granules for this stain. But among the orange spherules are some basophile granules, and with the centrifugal power noted above, some orange spherules may be found in the basophile band. But with a sufficiently high power a practically complete separation of basophile and acidophile granules may be brought about.

Attention must now be directed to a definite configuration in the distal or yellow hemisphere, viz., a clear, spongy area which extends into the base of the clear band, and which is surrounded distally and laterally by acidophile granules. It is similar to the central spongy area of the normal control eggs with which it must be identified. It contains relatively few granules, which are arranged in a coarse meshwork. The central end of this area is occupied by an aggregation of basophile granules which are so much more densely packed than those in the clear band, with which the special aggregation is continuous, as to contrast strikingly with it in sections; particularly with low powers of the microscope. The form of this aggregation is lunate or biconvex in section, thick in the center and diminishing towards the ends.

The large acidophile granules tend in a distal direction and the smaller basophlle granules in a central direction. If there were no differences in resistance in the ground substance they would pass in straight lines. But the fact is that they do not do so, and this is *prima facie* evidence of differences in resistance of the ground substance in different areas:

The basophile cap in the central boundary of the spongy area is a clear demonstration of this principle: In the first place it is an aggregation of basophile granules; this is proved by the staining reaction, always basic. That the stain is in the granules and not in the ground substance is readily demonstrated in thin sections, and the granules are of the same size as the basic granules of the clear band. In the second place, since such basophile granules tend to move centrally, that is, towards the hyaline band,

under the influence of centrifugal force, this aggregation, which does not exist in the control eggs, must have come from the distal hemisphere. But as they lie at the central end of the spongy area, and are most numerous opposite the center of this area, and diminish towards the sides, it is obvious that they must have come from the interior of this area.

They are in fact a swarm of microsomes moving under the influence of centrifugal force, but delayed on the borderland of the

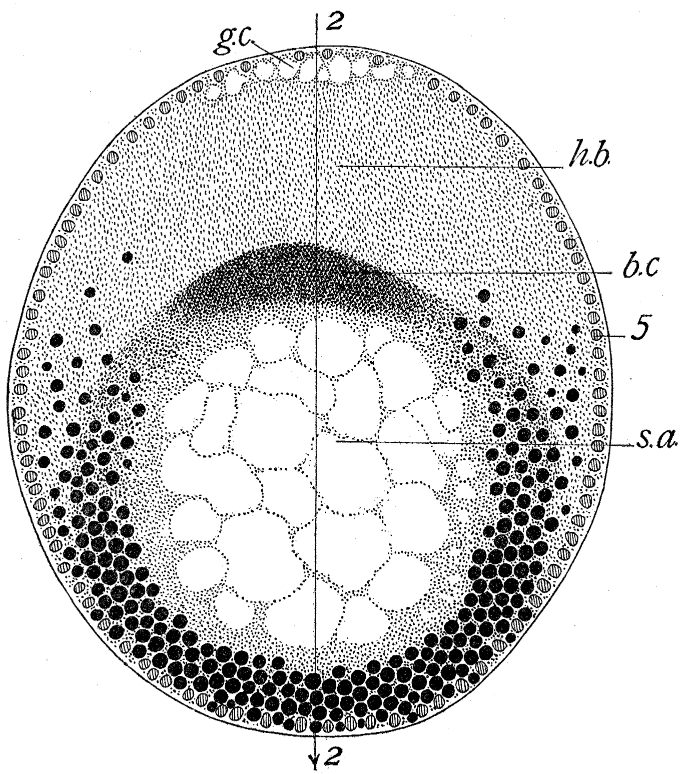

FIG. 7. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. The primary axis of the egg is unknown in this case. *2–2*, secondary axis; *5*, ectoplasmic zone; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area; stained in iron hæmatoxylin and orange *G*.

hyaline band (cf. Fig. 7), where they belong by virtue of their specific gravity, by the greater density (or other mode of resistance) of the wall of the spongy area.

This property of the wall of the spongy area also explains the

form of aggregation of the acidophile granules, which tend on this account to pass around and not into it. It also explains the more striking character of the spongy area in the centrifuged and the control eggs (cf. Figs. 6 and 4), because the granules within this area are free to segregate at its ends, but those without are prevented from entering.

Similarly, the persistence of the granules in the ectoplasmic zone indicates that this is another region of greater density of the ground substance. It requires a much greater centrifugal force to dislodge these granules than those lying within the egg, but when they are so dislodged they appear to be the heaviest class of granules within the egg, for they aggregrate at the distal pole (Fig. 9).

The following diagrams (Fig. 8) illustrate both the conception of the ground substance to which I have come as a result of the experiments, and the effect of the centrifugal force on the ground substance itself: The latter consists of four concentric layers, 2, 3, 4 and 5 (Figs. *A* and *C*); of which 5, the ectoplasmic stratum, is relatively dense, 4, the zone containing most of the acidophile granules of the normal egg is relatively fluid, 3, again, is a dense ring-shaped zone, surrounding 2, the central, more fluid spongy area.

The egg is an elastic sphere; it therefore undergoes elongation in the direction of the centrifugal force (Fig. 8, *B* and *D*); this is perfectly obvious immediately after centrifuging. The arrows in the figures to the left indicate the direction of the centrifugal force and the figure to the right in each case represents the result. Now observation shows that the spongy area of centrifuged eggs lies in the distal hemisphere, hence elongation of the ground substance must have taken place on the central side, owing presumably to the tension of the specifically very light substances (*e. g.*, fat globules) found here.

I have employed five or six different degrees of centrifugal force in examining this question and have made a considerable number of experiments with each. The configuration which we have just described varies somewhat with different degrees. With less force the same configuration is found down to 575 revolutions in 17 seconds with a radius of 13 cm. With higher forces

it tends gradually to disappear as is to be expected, for it is obvious that with a sufficient force the granules would be driven through or out of areas of greater density very readily and would segregate solely with reference to their specific gravity. With a

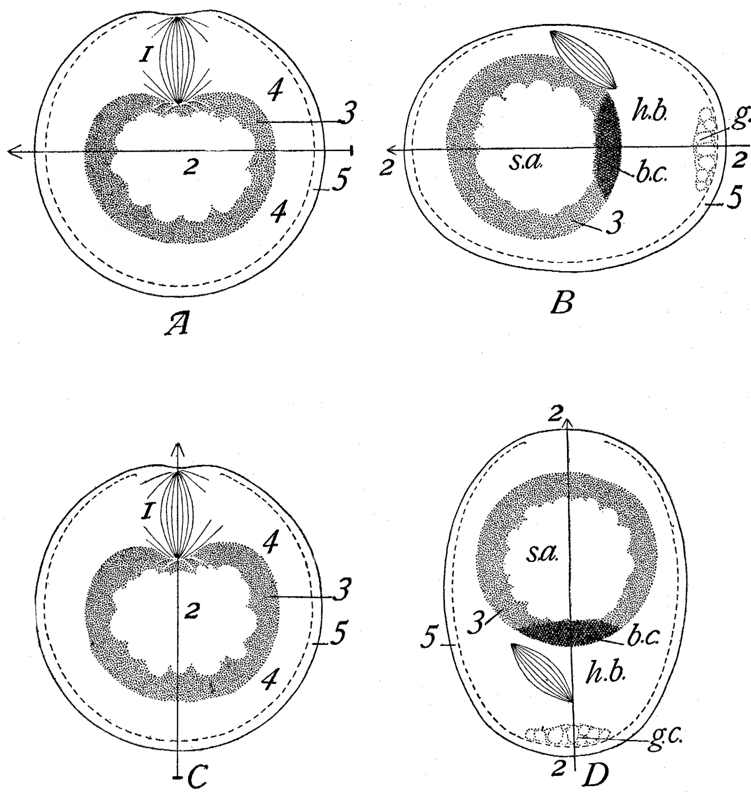

FIG. 8. Diagrams to illustrate structure of the ground substance of the egg of *Chætopterus* as revealed by centrifuging. *A* and *C*, controls: the arrow in each represents the direction of the centrifugal force. *B* and *D*, resultant conditions after centrifuging; *B* when the direction of the centrifugal force is as in *A*, *D* when the direction of the centrifugal force is as in *C*. *1*, spindle area; *2*, spongy area of control eggs; *2–2*, secondary axis of centrifuged eggs; *3*, dense ring-shaped zone in the ground substance; *4*, relatively fluid zone; *5*, ectoplasmic zone. The numbers have about the same significance as in Fig. 4. *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area of centrifuged eggs.

speed of 7,500 revolutions in one minute at a radius of about 6 centimeters, one can hardly observe any trace of the concentric arrangement (Fig. 9). Abundance of proof can be furnished for

these statements, but there would not be much value in multiplying instances by describing all of the intermediate conditions.

The same configuration is found in all stages of maturation and fertilization up to the first cleavage, at which time the cleavage naturally causes modification. Whether or not it reappears in each cell is a question that I have not examined.

It may be objected that the configuration shown could be produced as readily by mass movements of the protoplasmic areas as by individual granule movements. But such a conception would meet a fatal difficulty in the case of the central spongy area ; the basophile cap in the central end (with reference to the centrifuge) of this area could not be produced in any other way than by a granule movement. Moreover, detailed examination of the movements in the spherular zone (Fig. 4, *3*) seem to me to be equally conclusive : If the movements produced by centrifugal force in this area were mass movements of the protoplasm as a whole the conformation of this zone should vary with the direction of the centrifugal force until it reached a final position of equilibrium, for the reason that it would fold in different ways around the central spongy area. The graded series of centrifugal forces employed may be regarded as showing stages in the segregation of the granules of this area. If the movements were mass movements the animal pole extensions of the spherular zone (Fig. 4) must wrap around the central spongy area where the centrifugal force acts at any appreciable angle from the egg-axis. But this is not the case ; the proximal pole of the spongy area is never covered by such a fold. With the lowest speed, in directions at any appreciable angle to the egg-axis, one finds that the hyaline zone is dotted with granules of the spherular zone, evidently caught in migration ; such granules are rarer with successively higher powers of the centrifuge, until they are practically absent. It is obvious that they pass individually across the hyaline zone.

Another conclusive proof of individual granule movements is shown in Fig. 9. The spindle is in a very high degree resistant to penetration, and it therefore often acts as a block to migrating granules, which pile up against it. The figure illustrates the blocking of fat granules which are for the most part accumu-

lated at the central end of the egg (upper end in the figure). Innumerable cases of this kind are found in the preparations.

Again, as already noted, the ectoplasmic granules tend to keep within the ectoplasmic zone with low powers of the centrifuge.

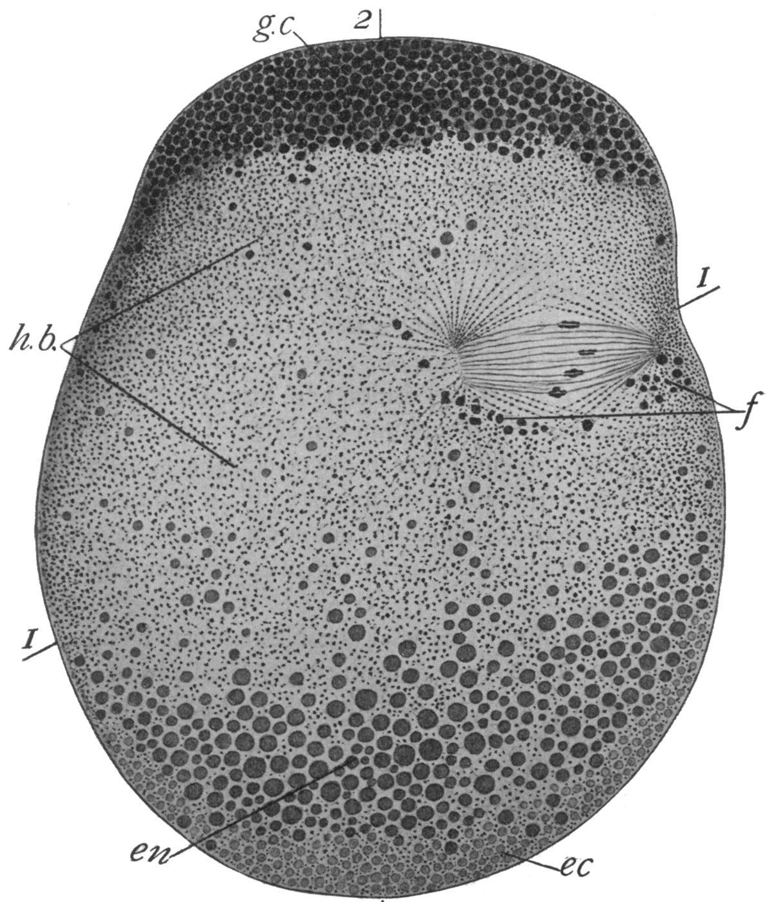

FIG. 9. Section of an egg of *Chætopterus* centrifuged 7,800 revolutions in one minute at a radius of 6 cm. The egg was killed in Flemming's fluid, and the fat granules of the gray cap are stained black. The spindle blocks fat globules passing in a central direction (*f*). *1–1*, primary axis; *2–2*, secondary axis; *ec.*, ectoplasmic spherules; *en.*, endoplasmic spherules in the distal hemisphere; *f.*, fat globules blocked by the spindle, *g.c.*, gray cap; *h.b.*, hyaline band.

But with higher powers they pass to the distal pole of the egg. Nevertheless the ectoplasmic zone may be recognized without

the granules, after fixation with Flemming's fluid, as shown in Fig. 9.

The conclusion of this whole matter is that there is demonstrative evidence of individual movement of all classes of granules under centrifugal power, and no evidence of mass movements of protoplasmic areas. These movements reveal a definite form of concentric organization in the ground substance, which is only indistinctly revealed by the granule arrangement seen in the normal egg.

It does not require much argument to show that the organization of the ground substance is the primary factor that determines the concentric arrangement of the granules. The idea that the organization in the ground substance is secondarily produced by a given arrangement of granules is contrary to the entire series of results contained in this paper.

The granules reach their position and form the normal segregation pattern by "flowing" movements. Unless the entire argument of the present paper is incorrect, the flowing movements are simply granule movements within a firmly organized ground substance, determined by unknown factors involved in the relations of nucleus and granules to each other and to the ground substance.

It is incredible to me that vital organization can be bound up in a flux of all protoplasmic constituents. Vital processes must be bound up with some firm condition of aggregation in the protoplasm.

But how, it may be asked, are polarity and bilaterality connected with the concentric organization of the ground substance revealed by centrifuging? The answer to this question is not at all obvious. These properties are shown by the experiments to be properties of the ground substance; and the experiments further show, as illustrated in Fig. 8, that the ground substance is merely distorted, not essentially altered by centrifugal force, and this furnishes a physical basis for the persistence of polarity and bilaterality in the original directions. But their nature is not in the least revealed by the experiments. The results only furnish a partial answer to the old question, how life and organization can exist in a total flux of materials, by showing that the flux is only partial.

Morgan has made the suggestion (*Science*, August 28, 1908, p. 288) that formative substances other than the visible granules here referred to may possibly be present in the sea urchin's egg, and that such materials are not seriously disturbed by a centrifugal force sufficient to separate the visible substances of the egg; but that if these more fundamental substances are displaced, interference with the normal development would be expected. According to this conception vital organization would still be a function of equilibrium and interaction of diverse substances. The logical possibility of such a conception must be admitted, but it avoids none of the difficulties inherent in the usual hypothesis of organization, and seems to me to offer no possibility for explaining the polarity or bilaterality of parts of an ovum or the phenomena of regulation in general.

The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions (as, for instance, when the yolk granules are packed closely into the small cell of the two-celled stage of *Chætopterus*) seem to me to argue for a molecular basis of the fundamental principle of vital organization.

Lehmann's observations on fluid crystals offer interesting analogies that are suggestive in the consideration of such fundamental biological problems. They show that the conception of crystallization as applied to vital organization does not meet with some of the difficulties formerly considered inherent in the analogy. Particularly his observations on the so-called myelin forms where innumerable fluid crystals determine the form and behavior of a single aggregate, suggest most interesting biological analogies, and aid to a certain extent in conceiving a doubly heteropolar condition of aggregation such as is presented by the animal egg.[1]

[1] It is not of course the author's intention in this communication to attempt to establish the validity of the analogy, but only to point out its possible application to the demonstrated organization of the ground substance of the cytoplasm. A good discussion of the entire analogy of crystallization to morphogenesis is contained in Przibram's article "Kristall-Analogien zur Entwicklungsmechanik der Organismen," *Archiv für Entw'mech.*, Bd. XXII., 1906.

The principle laid down here as to the molecular basis of cytoplasmic organization must apply not only to the stages considered but to all stages of development and to all parts of the organism.

The polarity of the egg and of living parts generally has been compared to magnetic polarity. Thus parts of a magnet exhibit polarity in the same direction as the original whole, and the north pole of the part is as true a north pole as the north pole of the whole magnet; the same is generally true of polarity in living things: a piece of *Hydra*, for instance, exhibits the same polarity as the entire animal of which it is a part. But push the comparison a little farther and the analogy appears to break down: a piece from the north end of a magnet has no more northness, so to speak, than a piece of the same size from the south end; on the other hand, an animal piece of an entire egg or an oral piece of an entire animal frequently has considerably more animal or oral properties than a piece from the vegetative or aboral end. The same difficulty of course arises if we use the analogy of crystallization. But it may be that it is due to secondary causes that modify the result without detriment to the validity of either analogy.

In conclusion a few words with reference to the so-called formative stuffs: So far as they are to be identified with the visible substances segregated by the centrifuge, it would appear to be indicated by the experiments that they can play no specific rôle in differentiation, because in centrifuged eggs they may occupy variable positions in the embryo. I believe, however, that the matter requires further investigation. It seems hardly credible that the strictly determinate distribution of these substances in normal ontogeny is meaningless, and that abnormal distribution is a matter of indifference. It is noteworthy that a large proportion of centrifuged eggs die before development has proceeded far, and that there is a distinct tendency towards abnormalities in the surviving eggs. It is natural to correlate these facts with unregulated abnormalities of distribution of granular materials. In any event a more careful study of the facts is needed.

It should be borne in mind that only the factors of polarity and bilaterality, the localizing factors in general, are shown to be functions of the ground substance. The respective rôles of

nucleus and granules are undetermined by these experiments; but their importance in the physiology of development is not open to question.

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